AstraZeneca

November 24, 1999

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

RE: *Docket No. 98D - 1195*
"Bioanalytical Methods Validation for Human Studies"

Dear Sir or Madam:

Reference is made to the January 5, 1999 Federal Register notice announcing the availability of a Draft Guidance for Industry entitled "Bioanalytical Methods Validation for Human Studies". Reference is also made to comments submitted to this docket by Astra Pharmaceuticals, L.P. on April 15, 1999.

At this time, we have some additional comments on the draft guidance which are attached.

Thank you for your consideration.

Sincerely,

Elizabeth Fenna
Senior Regulatory Project Manager
Regulatory Affairs

98D-1195

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"Guidance for Industry: Bioanalytical Methods Validation for Human Studies"

Background

This draft guidance was published for comment on January 5, 1999. Astra Pharmaceuticals LP submitted comments on this draft guidance on April 15, 1999. Since that time, there have been further discussions-regarding this guidance (e.g. at the Bioval '99 meeting in London, UK, 21-22 June 1999 arranged by the Royal Pharmaceutical Society, the Royal Society of Chemistry's Joint Pharmaceutical Analysis Group, the Pharmaceutical Sciences Group and the European Federation for Pharmaceutical Sciences). At this time, we have additional comments on this draft guidance.

General comment

The guidance at times is not clear and consistent and many terms used in the document need clarifying to avoid misinterpretation. Also, we recommend that some parts of the document need to be more specific. Our specific comments on the guidance are presented below.

Page/line number	Comments
Page 1	It is not clear why the proposed guidance only addresses studies in humans. We recommend that animal studies should also be covered by this guidance.
Page 2, line 13	Please clarify the difference between a "minor modification" and a "major modification".
Page 3, line 22	Please clarify the difference between "quality control samples" and "the analyte in spiked samples".
Page 3, line 31	We suggest the following change be made: Change from: "...from six individuals under controlled conditions, with reference to time of day, food ingestion, and other factors considered important in the intended study." to "...from six individuals under conditions considered important to the intended study, e.g. time of day, food ingestion, and other factors."
page 3, line 35	We suggest the following change be made: Change from: "The results should be compared to those obtained with an aqueous solution of the analyte at a concentration...." to "The results should be compared to those obtained with an adequately pure solvent of the analyte at a concentration...."

Page 4, section B. Calibration Curve	We recommend that this section be clarified to state that the number of calibration levels recommended in this section are used exclusively for defining the calibration characteristics. We believe that the same number of levels used for the calibration curve(s) may not necessarily be used later on during analysis of unknown samples in a study, as long as equal performance is demonstrated.
Page 4, line19	We suggest the following change be made: Change from: "A calibration curve should be prepared . . . of the analyte." to "A calibration curve should be prepared . . . of the analyte. However, other matrices may be used if equal performance is demonstrated."
'age 5, line 6	We suggest the following change be made: Change from: *"2. Linearity"* to *" 2. Calibration relation"* Calibration curves are seldom perfectly linear.
'age 5, section .. Linearity	Please clarify the following: 1. Are the acceptance criteria for the deviations given based on back-calculated values using a *single* determination? 2. What is the basis for these limits?
'age 5, line 15	We suggest that the following factor be omitted: "0.95 or greater correlation coefficient (r)" There is no direct relevance between (r) and method characteristics.

Page 5, line 20 and 30	Please clarify the following: The suggestions on precision (p. 5, l. 20 "A minimum of three concentrations in the range of expected concentrations is recommended.") and the suggestions on accuracy (p. 5, l. 30 "A minimum of five determinations per concentration should be conducted for a minimum of three concentrations in the range of expected concentrations." are not consistent with the suggested four concentrations on p. 6,1. 18, "...(2) LOQ quality control (QC) samples, (3) low QC samples, (4) medium QC samples, (5) high OC samples,..."
Page 5, line 24	We recommend that "..... or reproducibility" be changed to "..... or repeatability".
Page 6, line 7	Please clarify the following: The meaning of the wording "...pure authentic standard..." p. 6, l. 7. Is the meaning the same as for "...unextracted standards..." (p. 6,1. 13) and for "...reference standard..." (p. 6, l. 20 and 29)? Does this refer to pure solvent spiked with analyte or extracted matrix spiked with analyte?
Page 6, line 16	We suggest that the following change be made: Change from: "Each batch should contain... a reference standard." to "One of the batches should contain .... a reference standard. For the other two batches, the calibration curve should be omitted." In the pre-study validation, it would be more appropriate to test the accuracy and precision by spiking samples for the standard curve in one matrix batch and preparing QC samples in three different batches of matrix. In this way, interference from different matrices will be better taken into account.
'age 7, line 1	Please clarify the following: How should the calculations be performed?
Page 7, line 24	We suggest that the following change be made: Change from: "....should be determined using three freeze and thaw cycles..." to "....should be determined using the maximum number of freeze and thaw cycles expected in the study."

Page 7, line 27	We suggest that the following change be made: Change from: ″. . . .24 hours and thawed unassisted at room temperature.. .″ to ″. . . .24 hours and thawed according to protocol.. .″
Page 7, line 27	We suggest that the following change be made: “When completely thawed.. .kept refrozen for 12 to 24 hours.” to . ..” When completely thawed.. . kept refrozen for at least 12 hours”. The issue here is that the samples are refrozen, not that they are thawed again the day after.
Page 8, line 2	We suggest that the following change be made: Change from: “Three aliquots of each.. .should be thawed at room temperature and kept at this temperature from 4 to 24 hours.. .in the intended study) and analyzed (Buick 1990).″ to “Three aliquots of each.. .should be thawed and kept at the expected temperature for the expected period of time according to protocol.”
‘age 8, line 13	We suggest that the following change be made: Change from: “The concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing (Buick 1990)” to “The concentrations of all the stability samples should be compared to the mean of back-calculated values from the first day of long-term stability testing.”
‘age 8, line 19	We suggest that the following change be made: Change from: “The stability of stock solutions of drug and the internal standards should be evaluated at room temperature for at least 6 hours. The stability samples should then be refrigerated or frozen for 7 to 14 days or other relevant period” to “The stability of stock solutions of drug and the internal standards should be evaluated at the relevant temperature for a relevant period of time according to protocol ″

Page 10, Line 10	We suggest that the following sentence be omitted: "All study samples from a subject should be analyzed in a single run." If the accuracy and precision of the method is within the acceptance criteria, then this recommendation seems unnecessary.
Page 10, line 18	We suggest the following change be made (two alternatives): Change from: "At least four of the six QC samples should be within ± 20% of their respective nominal value. Two of the six QC samples may be outside the ±20% of their respective nominal value, but not both at the same concentration." To first alternative : "At least three of the six QC samples should be within ±20% of their respective nominal value. Three of the six QC samples may be outside the ±20% of their respective nominal value, but not two at the same concentration." second alternative : "At least five of the six QC samples should be within ±40% of their respective nominal value. One of the six QC samples may be outside the ±40% of their respective nominal value." A suggestion on acceptance criteria of ± 20% of their respective *nominal* value, is inconsistent with the Precision criteria, p. 9 1.7, in *combination with* the Accuracy criteria, p. 9 1.9. For a single QC sample, it is not possible to distinguish between combined effects of deviation caused by Precision or Accuracy. There is a 33% chance for a QC sample to be ≥ 20% off the nominal value with a CV of 20%, for a method with an Accuracy of 100%. An Accuracy of 80 or 120% would yield a 50% chance for a QC sample to be ≥ 20% off the nominal value.
'age 10, line 30	We suggest that the following sentence be omitted: "Reassays should be done in triplicate." We believe that specific requirements for reassays should not be addressed in this guidance but should be based on good science and appropriate to the situation.

Page 10, line 31	We suggest that the following change be made: Change from: "The pre-study validation experiments, the data generated from them, and the assay quality control data should be recorded in a bound laboratory notebook. The entries should be signed by the chemist and witnessed by the laboratory supervisor." to "The pre-study validation experiments, the data generated from them, and the assay quality control data should be recorded according to cGMP/GLP regulations and the Organization for Economic Cooperation and Development (OECD) guidelines."
Page 11, line 11 and line 13	We suggest that the following sentence be omitted: "Calibration curves used in analyzing samples and intra-day accuracy and precision data. " If the acceptance criteria for analysis are based on QC samples the calibration curves and statistics of the calibration samples would be of no interest for the in-study validation. If still required, we suggest that back-calculated values for the calibration samples be sufficient.
Page 11, line 18	We suggest that the following change be made: Change from: "Reasons for missing samples" to "Reasons for missing results for analyzed samples"
'age 11, line 25	We suggest that the following change be made: Move, "Calibration curves, equations, and weighting factors used, if any" to the section "Documentation for pre-study validation should include:" p. 11.
'age 12, line 3	Please clarify the following: 1. Which SOPs are referred to here? 2. What raw data would be required here? The way that calculations of concentration are performed would already be documented in the pre-study validation data. 3. The meaning of "Reassay sample sets".

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